The Comparison of Salivary Alpha Amylase Enzym Level Between Anxiety Patients and Depression Patients

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Abstract

Salivary α-amylase (sAA) serves as a marker of sympathoadrenal medullary system (SAM) activity. Research on the comparison of salivary α-amylase levels between anxiety disorder and depression disorder had not been widely reported. In the current study, 30 anxiety patients, 30 depression patients and 30 healthy volunteers were assessed with Hamilton Anxiety Rating Scale (HARS) for anxiety patients and Hamilton Depression Rating Scale (HDRS) for depression patients. The measurement of the salivary α-amylase (sAA) was performed before the anxiety patients and the depressed patients received treatment. Salivary α-amylase (sAA) of the anxiety and depression patients group increased significantly compared to the control group (healthy people). There was a significant correlation between the scores of HARS and of HDRS to the level of sAA enzyme. Regression analysis indicated a potential increase of the sAA level in the amount of 5.673 kU/L per one score of HARS and one additional score of HDRS also potentially increased in the amount of 0.925 kU/L of the sAA enzyme level. Additionally, R^2 obtained from linear regression for anxiety patients group was 0.442 which meant that the effect of HARS score on sAA was 44.2% and 55.8% sAA rate was influenced by other variables. R^2 for depression patients group was 0.457 which meant that the effect of HDRS score to the level of sAA enzyme was 45.7 % and 54.3 % was influenced by other variables.

Keywords: anxiety; depression; salivary alpha amylase.

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1. Introduction

Anxiety and depression disorders are common psychiatric disorder. They are associated with an increased in morbidity and mortality rates. The prevalence rate of anxiety and depression is 6% for the age of 15 years old or about 14 million people [1].

The high prevalence rate of mental disorders lately is estimated due to the higher stress level. The biopsychosocial approach explains the interaction among biological, psychological and socio-cultural factors in the occurrence of mental disorders are explained in the diathesis-stress model. According to this model, individuals who have a particular susceptibility to mood disorder when exposed to a stressor which is not sufficient to normal individu, will indeed suffer these disorders [2, 3].

In the event of stress, physiologically the body will produce a series of coordinated responses to keep the body homeostatis. Stress will activate both the body stress system of the hypothalamus pituitary adrenal (HPA) axis and the sympathetic adreno medullary (SAM) system. Currently several biomarkers have been widely used as a marker of the reaction of physiologic stress systems, including cortisol through the HPA axis pathway and some catecholamine plasmas such as epinephrine through the SAM system [4-6].

Recently, the research about sAA enzyme on various mental disorders has been improved, many studies showed the role of sAA enzyme as biomarker of the sympathetic nervous system. As it is well known that salivary alpha amylase enzyme is one of the most important enzymes contained within saliva, this enzyme acts in the digestive process of macromolecules such as carbohydrates and starches and also play a role in the process against bacteria [7]. Saliva production and secretion include complex biological processes. Saliva is produced through three major glands: the parotid gland, the submandibular glands and the sublingual glands each of which is located in the left and right pairs in the oral cavity. sAA enzyme is produced about 80% in the parotid gland. Salivary glands are made up of various cell types, namely, acinar cells, ductal cells and myoepithelial cells. Cells are conserved by both the sympathetic and parasympathetic nerve branches of the autonomic nervous system [8]. Increased levels of sAA enzyme are associated with increased activity of the activated sympathetic adreno medullary (SAM) system at the time of stress. The sAA enzyme is produced directly by the salivary glands, all through the stimulation process of the sympathetic nerves that conserve the salivary glands therefore the salivating factors of saliva can also influenced sAA enzyme level. The things that can influenced the activity of the sAA enzyme are : age, which is very low and undetectable in the newborn's basal activity, smoking can suppress acute activity of the sAA enzyme and smokers chronic generally show low levels of sAA, some studies have found low sAA levels in chronic alcohol drinkers, drug agonists and adrenergic antagonists strongly update sAA activity, caffeine can stimulate sAA activity, exercise may increase the levels of sAA enzyme [9].

Biological markers (biomarkers) in a stressful state are useful clues in clinical practice of psychiatry as well as research. The difficulties of these biomarker measurements are mostly due to the sampling method which generally obtained through venous blood punksi. The process of this vein punksi can cause pain or stress which could affect the acute release of the catecholamine hormone and cause an increase in sAA enzyme level and in turn can cause bias to the measurement results. Measuring the level of sAA enzyme through saliva is non

invasive, it does not make the patient or research subjects to stress as it gives ease in taking data and comfort to patients or research subjects, besides time needed is relatively short, easy to do anywhere and anytime [9].

A previous study by Inagaki and his colleagues (2010), showed a significant increase in salivary alpha amylase enzyme levels in schizophrenic patients compared with control group [10]. Research on depression patients by Tanaka and his colleagues (2012), showed higher levels of sAA enzyme in depressed patients who had been undergoing therapy and then given electric shock stimulation than before being stimulated [11]. Study by Fisher and his colleagues (2010) showed, sAA enzyme levels were higher in anxiety patients after given acute stress stimulation. In contrast study by Bagley and his colleagues (2011) showed no difference in sAA enzyme level between the depression patients and control groups. The above studies largely show higher levels of sAA enzyme in patients who were given acute stress. Previous studies were mostly performed on patients who had received therapy and were given acute stress again, and only a few studies examined levels of sAA enzyme in psychiatric patients who had never received any treatment. The researchers were interested to know the level of salivary alpha amylase enzyme especially in naive-drug anxiety and depression patients in which might be associated with the severity of symptoms of the disorder. To our knowledge, this research has never been conducted before. Based on this background, this study aimed to determine the ratio of salivary alpha amylase enzyme levels of patients with anxiety and depression and its association with the severity of symptoms of anxiety and depression.

2. Methods

2.1. Participants

The subjects were divided into three groups, the anxiety group, the depression group and the control group. The subjects were recruited at the psychiatric polyclinic of Wahidin Sudirohusodo Hospital. The subjects in this study were all patients who met the diagnosed criterion of anxiety disorders and depression disorders based on ICD-10. All subjects were 18-45 years old. Patients suffering from chronic metabolic disease and disease of the oral region were excluded. The control group (healthy people) were teachers in elementary school, 18-45 years old. They had no history of mental disorder, chronic metabolic and oral disease. All subjects were provided with complete written and oral descriptions of the study, written informed consent was also obtained. The protocol was approved by the local ethics committee.

2.2. Design and procedure

This research was an observational analytic research with cross sectional design. The independent variables of this study were anxiety disorder and depression disorder. The dependent variables were the salivary alpha amylase (sAA) enzyme. Subjects data who met the inclusion and exclusion criteria were collected including name, full address, gender, age, ethnicity, religion, last education, occupation and marital status. The subjects were anxiety and depression patients diagnosed by ICD-10 criterion and had never taken any medication. After recording the identity of the subjects, the researcher determined the severity anxiety and depression symptoms by using HARS and HDRS.

2.3. Measurement

2.3.1. Sampling Methods and biochemical analyses

Saliva was collected by using cocoro meter (Nipro Co, Japan), consisting of a disposable test strip that used reagent paper and a monitor. The test strips was inserted into the oral cavity sublingually for about 30 seconds, then the test strip was inserted into the monitor which revealed the nominal of sAA enzyme levels. It took only 1 minute to measure the sAA enzyme level. The measurements of sAA levels were performed betwen 9 a.m and 2 p.m. The measurements were conducted at least 2 hours after the last meal to minimize the effect of food or drink on salivary alpha amylase enzyme activity and the subjects were expected to rest about 10 minutes before starting the test. The measurement of salivary alpha amylase enzyme by this tool using optical detection method. The substrate (saliva) was collected using strip which contain a strip reagent so that if mixed : Gal-G2 (Galaktospyranosylmatosa) as the substrate will bind to CNP (Chloro-Nitrophenyl) chromogen compound to become Gal-G2-CNP (2-Chloro-4-nitrophenyl-4-O-ßD-galactospyranosylmaltoside). This 2-Chloro-4-Nitrophenyl (CNP) reagent strip will hydrolyze the substrate to detect the salivary alpha amylase enzyme by producing a yellow product. GAL-G2-CNP →AA GAL-G2 + CNP (white to yellow). Changes in the intensity of this color were in accordance with changes in activity of the salivary alpha amylase enzyme, the higher the intensity of the yellow color meant the higher the sAA enzyme levels. Then the reagent strip was inserted into the optical analyzer, in which the device was capable of measuring the activity of salivary alpha amylase enzyme. The reference value of the sAA enzyme levels using the portable cocoro meter monitoring device is 0 - 30 kU / L = normal, 31 - 45 kU / L = mild stress, 46 - 60 kU / L = medium stress and > 60 kU / L = severe stress [12].

2.3.2. Psychometric measures

To examine the level of anxiety and depression symptoms of the subjects, the following rating scales were used :

- Hamilton Anxiety Rating Scale (HARS) : perceived anxiety was assessed. The HARS comprises 14 subscales, feelings of anxiety, tension, sleep disturbances, intelligence disorders, fear, depression, somatic symptoms, cardiovascular symptoms, respiratory symptoms, gastrointestinal symptoms, urogenital symptoms, autonomic symptoms and behavior during interviews.
- Hamilton Depression Rating Scale (HDRS) : perceived depression was assessed. The HDRS comprises 21 subscales, feelings of guilt, tendency of commiting suicide, initial insomnia, middle insomnia, late insomnia, work and activity, daily activities, agitation, psychic anxiety, somatic anxiety, somatic digestive symptoms, common somatic symptoms, genitals, hypochondriasis, weight loss, insight, daily variations, depersonalization and derealization, paranoid symptoms, symptoms of compulsive obsessions.

2.3.3. Statistical Analysis

The data was analyzed using statistical program of product of social science (SPSS) and presented as tables and

graphs. The data was tested for normal distribution and homogenity of variance using a kolmogorov smirnov and levene's test before the statistical procedurs were applied. Mann-whitney test was used for comparison among groups. The correlation was tested by spearman test.

3. Results

3.1. Sample characteristic

Ninenty subjects participated in the study. The consecutive resulted in three groups which were the anxiety disorder group, the depression disorder group and the control group (healty people), each group consist of 30 subjects.

Table 1: Demographic Characteristics

Characteristic		Frequency (n)	Persentage (%)
Sex	Male	39	43.3
	Female	51	56.7
Age (years)	18-26	9	10
	27-35	23	25.6
	36-45	58	64.4
Education	Elementary	7	7.8
	Junior High School	27	30.0
	High School	39	43.3
	Bachelor	15	16.7
	Postgraduate	2	2.2
Occupation	Employed	46	51.1
	Unemployed	44	48.9
Married	Married	62	68.9
	Not Married	28	31.1

Source : Primary data, 2017

Subjects proportion in terms of sex were more female (56.7%) than male (43.3%). In terms of age, they were divided into three age groups: (1) 18-26 years old, (2) 27-35 years old and (3) 36-45 years old. Most subjects was in the group of 36-45 which was 58 subjects (64.4%), group 27-35 was 23 people (25.6%) and group of 18-26 was 9 people   (10.0%). Based on the educational status, the subjects were divided into 5 levels of education which were graduate of elementary school, junior high school, high school, bachelor and postgraduate. The proportion of elementary school graduate was 7.8%, of junior high school graduate was 30 %, of high school graduate was 43.3%, of bachelor graduate was 16.7% and of post graduate was 2.2%. Most subjects were in high school. The proportion of subjects occupation was almost the same in both groups, 51.1% for employed

and 48.9% for unemployed. The proportion of subjects marital status was 68.9% for married and 31.1% for unmarried. It was obvious that the number of subjects in the married group was more than unmarried group.

3.2. Salivary Alpha Amylase respons

The Comparison of sAA enzyme level of anxiety disorder group and control group showed a significant difference between sAA enzyme level of anxiety patients (63.57 ± 32.22) and the control group (20.20 ± 7.81) with $p=0.000$ (see Figure 1.A). The comparison of sAA enzyme level of depression group (35.17 ± 11.92) and control group (20.20 ± 7.81) who showed a significant difference between sAA enzyme level of depression group and control group with $p=0.000$ (see Figure 1.B). Additionally, the comparison of sAA enzyme level in anxiety group and depression group showed a significant difference, in which the level of sAA in the anxiety group was higher than the depression group, with $p=0.000$ (see Figure 1.C).
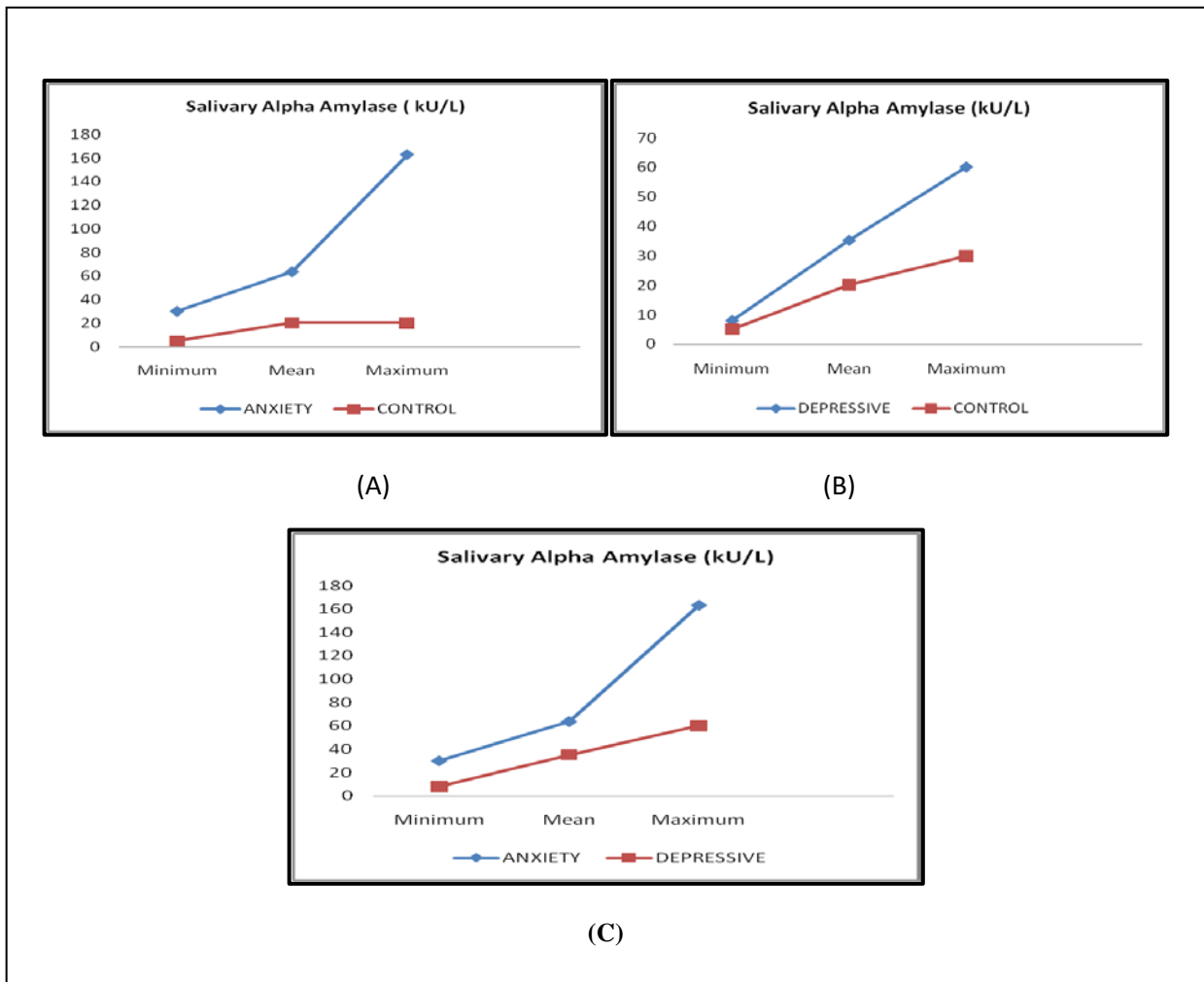

Figure 1: The comparison of salivary alpha amylase enzyme level. A : Anxiety subjects group and control group.   B : Depressive subjects group and control group. C : Anxiety subjects group and depressive subjects group.

In addition, spearman correlation test was used to asses the relationship between the sAA enzyme level and HARS score in the anxiety group showed a significant correlation ($p = 0.000$) with positive coefficient correlation and the correlation strength was high enough (0.854) in which an increase in HARS score was followed by an increase in the sAA enzym level.

We also analyzed the relationship between sAA enzyme level and HDRS score in the depression group showed a significant correlation ($p = 0.000$) with positive coefficient correlation and correlation strength was high enought (0.676) in which an increase in HDRS score was followed by an increase in the sAA enzym level.

Furthermore, to find out whether the HARS score significantly influenced the sAA enzyme level, a linear regression test was used, the results can be seen in table 2, the value of R which was the symbol of the correlation coefficient value was 0.665 which meant that the relationship between HARS and sAA enzyme levels was quite strong. R^2 value was 0.442 which meant that the effect of HARS score to the level of sAA enzyme was 44.2% and 55.8% was influenced by other variables.

Table 2: Regression analysis of improvement of anxiety symptoms represented by HARS score to sAA level

Model	R	Adjusted R Square	Unstandardized Coefficients		Standardized coefficient	Sig
			B	Std. Error	Beta	
(Constant)*	0.665	0.422	2.890	13.645	0.665	0.000
HARS score			2.783	0.951		

* Dependent variable: sAA level

To know the effect of HARS score on the level of sAA enzyme in this study, we analyzed by using linear regression equation, $Y = a + b X$. The Y is the dependent variable that is the level of sAA enzyme, and the X is the independent variable that is the HARS score.

Regression analysis calculation come up with the constant number (a) for sAA level that was 2.890 and the HARS score (b) was 2.783. The regression equation for sAA level was $Y = 2.890 + (2.783) X$, which meant if the HARS score increased by one point, the level of the sAA enzyme would be raised 5.673 (see Figure.2).

Additionally, the regression test for HDRS score to the level of sAA enzym showed an adjusted R^2 value of 0.457 which meant that there was still a considerable percentage of other variables influenced the level of sAA enzyme up to 54.3 % (See Table 3).
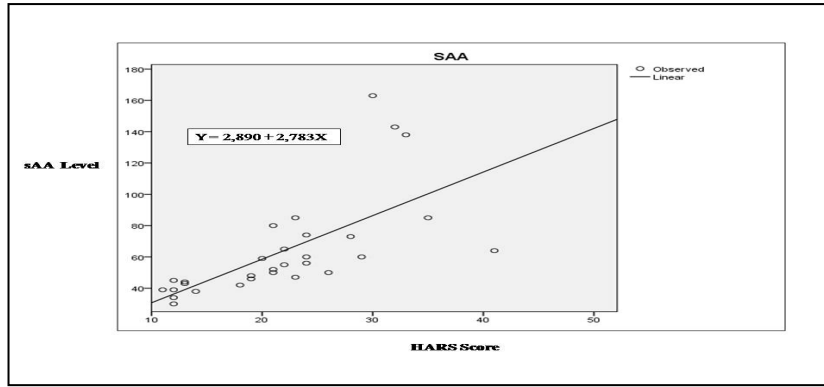
Figure 2: Linear correlation between improvement of anxiety symptoms represented by HARS score and sAA level.

Table 3: Regression analysis of improvement of depression symptoms represented by HDRS score to sAA level

			Unstandardized Coefficients		Standardized coefficient	
						Sig
Model	R	Adjusted R Square	B	Std. Error	Beta	
(Constant)*	0.676	0.457	-0.849	7.602		0.676	0.000
HDRS score			1.774	0.366		

* Dependent variable: sAA level

Regression analysis calculation come up with the constant number (a) for sAA level was -0.849 and the HDRS score(b) was 1.774. The regression equation for sAA level was Y = -0.849 + (1.774) X, which meant if the HDRS score increased, the sAA enzyme level would raise 0.925 in turn (See Figure.3).
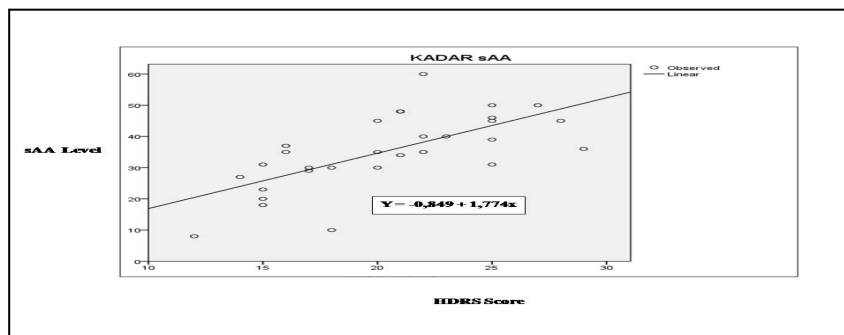

Figure 3: Linear correlation between improvement of depression symptoms represented by HDRS score and sAA level

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4. Discussion

In this study, there were a significant difference of the sAA enzyme level between anxiety group and control group ($p=0.000$), depression group and control group ($p=0.000$) and anxiety group and depression group ($p=0.000$). The mean value of the anxiety group was 63.57 kU/L, the depression group was 35.17 kU/L and the control group was 20.20 kU/L, normal level of sAA enzyme was < 30 kU/L. Based on the above result, the increased sAA level was more significant in anxiety group because in anxiety disorder, the state of stress was more acute and the sympathetic adrenal medullary (SAM) system was activated. The activation of SAM system would stimulate adrenal medulla catecholamine release of epinephrine and norepinephrine which would activate salivatorius gland resulting in increased secretion of sAA enzymes. In contract, for depression disorder, the stress tends to be chronic so that the SAM system wass less activated. In contrary, the HPA axis played a role in depression disorder so the increased sAA enzyme was relatively smaller [4].

Some studies support that the sAA may be a useful indicator for activity of the sympathetic nervous system. Salivary alpha amylase response can serve as an index for pathological disregulation of the autonomic nervous system (ANS) in specific clinical and subclinical conditions. Anxiety disorder was associated with autonomic changes. Salivary alpha amylase measurement might provide additional information about autonomic changes occurring in anxiety disorder.

The minimum level of sAA enzyme of anxiety group was 30 kU/L and the maximum level was 163 kU/L so the standard deviation obtained was quite large that is 32.221 kU/L. This difference could be caused by the sampling of sAA enzyme, not basal sAA level, in which it was performed at the psychiatric clinic at various times for each sample from 9 a.m to 2 p.m, other factors could affect the sAA enzyme levels, including waiting fatigue, hungry conditions, imagining or smelling cooking, these could lead to bias on the results of this study.

The results of this study were similar to previous research that has been done by Tanaka Y and his colleagues 2012 in patients with panic disorder, the results showed the increased levels of sAA enzyme in patients with panic disorder both in the basal state and after being given acute stress stimulation. The same results were obtained in several studies reported by Schumacher S and his colleagues 2013 [13]. One of the research conducted by Veen JF and his colleagues 2008 in anxiety disorders, showed increased levels of sAA enzyme in anxiety sufferers who were given acute stimulation [14].

In depression disorder, the increased levels of sAA were relatively smaller with the average value of sAA enzyme level of 35.17 kU/L. Stress that occur tended to be chronic so the SAM system was less activated. In contrary, HPA axis pathway acted more in depression disorders. Schumacher S and his colleagues 2013 reported several studies of sAA enzyme levels in depressed patients, whose result was consistent with this study, including a study by Bagley and his colleagues 2011 with no difference in sAA enzyme levels between depression and control group. Study by Girr M and his colleagues 2010 in major depression patients with suicide risk showed relatively small difference between patients and controls. However, different result was reported in a study by Tanaka Y and his colleagues 2012 in depression patients in basal state prior to acute stress stimulation, showed a significant increase in sAA enzyme levels with an average value of 60 kU / L.

We also examined the correlation of sAA level with HARS score and HDRS score. We found that there was significant correlation between the HARS / HDRS score and the level of sAA enzyme, which meant that an increase of the HARS/HDRS score would be followed by an increase in the sAA enzyme level.

The results of this study was similar to those of previous studies, so further research is needed to look at the sAA enzyme levels in various mental disorders with a higher sample size and basal sAA enzyme sampling for eliminating various confounding factors that might cause bias on the results of the study. Hopefully continuous research might further confirm the role of salivary alpha amylase enzymes as biomarkers of the activated sympathetic adrenal medullary system in stressful situations. In the future, objective examination of sAA enzyme levels was expected to be an additional routine that could help determine stress levels and monitoring the progress of patient therapy.

5. Conclusion

In conclusion, the level of salivary alpha amylase in anxiety disorder group was significantly higher than the depression disorder group and control group (healthy people) and the sAA enzyme level in depression disorder groupwas significantly higher than the control group. The increased HARS score of anxiety disorder was followed by a significant increase in sAA enzyme levels. Additionally, the increased HDRS score of depression disorder was followed by a significant increase in sAA enzyme levels.

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